Abstract

In rapidly proliferating cancer cells, endoplasmic reticulum (ER) tends to be overloaded with unfolded and misfolded proteins due to high metabolic demand. With the limited protein folding capacity of ER, cancer cells suffer from Unfolded Protein Response (UPR) stress and subsequently elevate autophagy, in which the protein-aggregates are degraded and provide free energy in form of amino acids allowing cancer cells for an uninterrupted proliferation. Consequently, several anticancer molecules targeting UPR-autophagy network are currently under clinical trials. Epstein-Barr virus (EBV) is associated with a number of B-cell lymphomas. EBV can readily transform quiescent B-lymphocytes into continuously proliferating lymphoblastoid cell lines (LCLs), providing an excellent model system for studying EBV associated B-cell lymphomagenesis. EBNA3C, one of the essential viral oncoproteins for B-cell transformation, manipulates multiple cell pathways – most strikingly cell-cycle/apoptosis and ubiquitin-targeted protein-degradation machineries. Interestingly, several components of these pathways were previously shown to be intricately connected with the UPR-autophagy network in the development of human malignancies. In our study, we have shown that EBNA3C transcriptionally upregulates several autophagy genes (ATG3, ATG5, and ATG7), particularly involved in autophagosome formation, under nutrient deprived conditions. EBNA3C recruits several histone activation epigenetic marks to regulate autophagy gene transcription. Moreover, EBNA3C specifically elevates PERK-eIF2α-ATF4 UPR signaling cascade in normal growth conditions, whereas in the presence of UPR-inducers it blocks further UPR activation, indicating EBNA3C promotes an adaptive condition for cell survival. Overall, our study provides a new role of an essential EBV oncoprotein in regulating autophagy-UPR cascade and offers novel targets for potential therapeutic expansion against multiple EBV induced B-cell lymphomas.

CONCLUSIONS AND PERSPECTIVE

4. Conclusions and Perspective

Although UPR-autophagy network has been shown to be critically involved in the development of multiple cancers, unfortunately little is known in tumor-virus associated cancers. The work was initiated with specific aim is to decipher potential role of this network in EBV associated B-cell lymphomas. EBV, the first human virus identified, has been shown to be associated with a number of B-cell lymphomas, particularly those are generated from immunocompromised background such as post-transplant and AIDS patients. *In vitro*, EBV can transform quiescent B-lymphocytes into continuously proliferating lymphoblastoid cell lines (LCLs), which express a full repertoire of viral latent genes. In laboratory settings, LCLs offer an excellent model system to study EBV induced B-cell lymphomagenesis and to understand the role of EBV encoded latent oncoproteins. Using genetic engineering, five antigens including four nuclear proteins - EBNA2, EBNA3A, EBNA3C, EBNALP along with a membrane associated protein, LMP1 were shown to be indispensable for *in vitro* B-cell transformation[13]. EBNA3C plays a critical role in B-cell transformation subsequently B-cell lymphoma development through manipulating multiple cellular pathways – particularly cell-cycle and apoptosis pathways through engaging transcriptional/post-transcriptional control as well as recruiting ubiquitin-mediated proteasomal degradation machinery[134]. Interestingly, a number of important proteins in these pathways can directly or indirectly regulate UPR-autophagy network. Given the multifaceted role of EBNA3C, we aimed is to develop a comprehensive model for the role of EBNA3C in regulating UPR-autophagy network during B-cell lymphomagenesis and provide potential targets for future therapeutic enhancement against EBV associated B-cell lymphomas[116].

In summary, our study suggests a model in which EBNA3C enhances autophagy by transcriptional upregulation of a number of autophagy genes specifically required for autophagosome biogenesis to cope with several cellular insults such as growth factor deprivation as well as prolonged ER-stress (Fig. 6.1.). Our study demonstrates a number of crucial findings which include:

- EBNA3C, an essential EBV oncoprotein, elevates the basal level of autophagy, which is further elevated under growth limiting conditions.

- EBNA3C mediated autophagic induction serves as a prerequisite for an uninterrupted cell proliferation in normal growth conditions.
- EBNA3C protects cell death induced by autophagy inhibition. While knockdown of ATG5, an essential component of autophagy machinery, sensitizes cell death of EBNA3C expressing B-cells in response to autophagy inhibitors, does not affect thapsigargin induced apoptotic cell death.
- EBNA3C transcriptionally upregulates the autophagy machinery specifically required for autophagosome biogenesis. Under growth limiting conditions, EBNA3C not only promotes transcription of genes in the autophagic program (viz. ATG3, ATG5 and ATG7), but also facilitates expressions of several death regulatory elements such as DAPK1, CDKN1B and CDKN2A.
- Reanalyzing the ENCODE ChIP-sequencing data followed by validation via ChIP-PCR demonstrate that EBNA3C recruits epigenetic marks associated with histone activation including H3K4me1, H3K4me3, H3K9ac and H3K27ac leading to transcriptional activation of several autophagy gene loci.
- EBV transformed LCLs are sensitive to autophagy-inhibitors (chloroquine and bafilomycin) and UPR-inducers (thapsigargin and tunicamycin) - a clue to potential therapeutic option against multiple EBV-linked lymphoproliferative disorders. As similar to autophagy inhibition, EBNA3C protects cell death induced by prolonged UPR induction.
- EBNA3C elevates basal level of UPR through specifically up-regulating PERK-eIF2α-ATF4 signaling cascade in normal growth conditions. However, in the presence of UPR-activators it blocks further UPR activation, indicating EBNA3C promotes an adaptive condition for cell survival.
- LC-MS/MS analyses of immunoprecipitated EBNA3C from EBV transformed LCLs in response to either proteasomal or autophagy-lysosomal inhibition reveal a comprehensive panel of novel cellular binding partners. EBNA3C interacts with many important cell machineries including RNA biogenesis, nucleosome formation, transcription factors and proteins involved in glycolysis and energy production. Moreover, proteasomal inhibition significantly reduces the EBNA3C interactome, while lysosomal inhibition enhances EBNA3C interacting cellular partners.

Overall, our results provide further insights into EBNA3C mediated viral oncogenesis, in which UPR-autophagy network plays an essential role in B-cell survival and thereby promoting B-cell lymphomagenesis. The current study offers novel molecular targets within the UPR-autophagy network for future therapeutic expansion against EBV associated multiple B-cell lymphomas[1]. This work would be immensely helpful for tumor virology field that how a viral oncogene subverts essential cell mechanisms. We certainly hope the study would draw attention of overall cancer biology field, where UPR-autophagy network appears to be one of the major areas for future drug development against multiple human cancers, both of lymphoid and epithelial in origin.

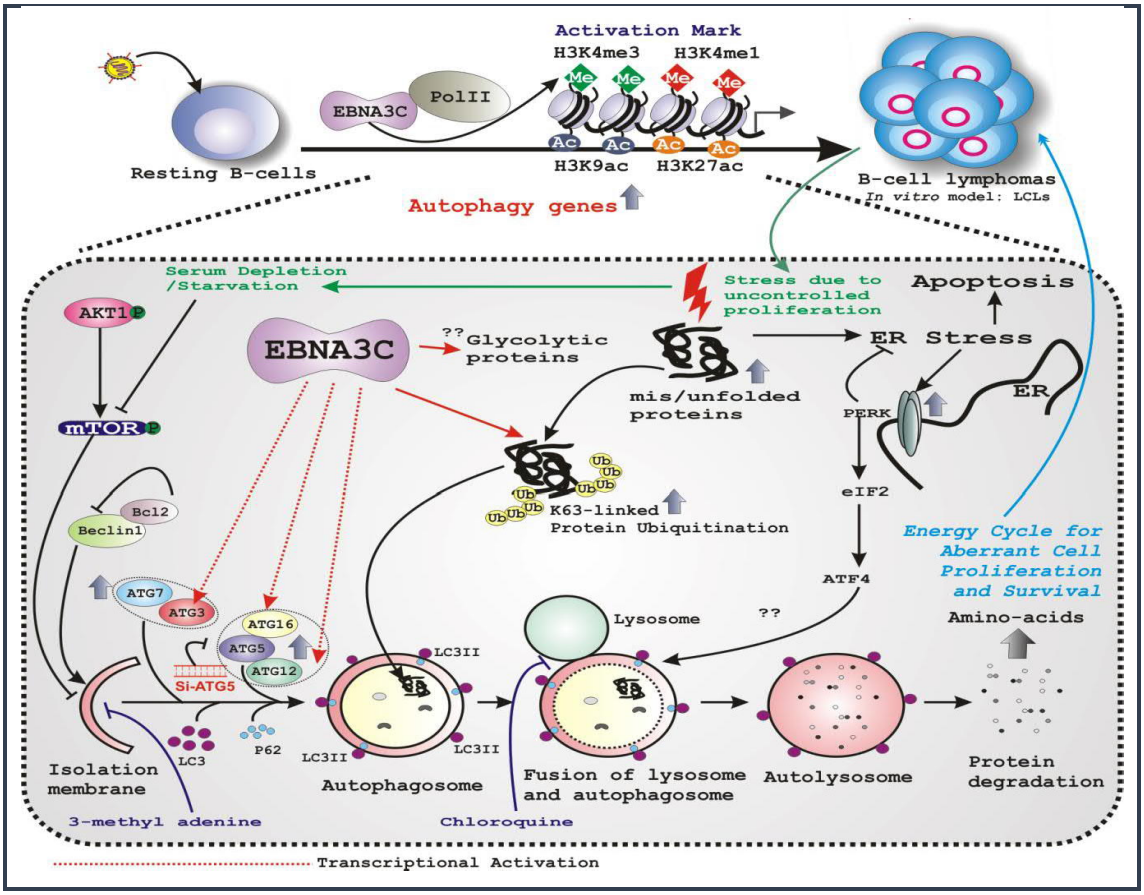

Fig 6.1. Model depicts EBNA3C deregulates autophagy-UPR network for B-cell survival. Schematic representation of EBNA3C induced autophagy. Upon EBV infection in resting B-cells EBNA3C recruits histone activation chromatin marks (H3K4me1, H3K4me3, H3K9ac and H3K27ac) to elevate a number of autophagy related gene transcriptions such as *ATG3*, *ATG5*, *ATG7*, *BCL2* and *EIF2AK3*. ATG3, ATG5 and ATG7 assist in autophagosome formation through inducing LC3-lipidation. BCL2 is an anti-apoptotic protein, which blocks Beclin-1 activity through direct protein-protein interaction, providing a feedback regulation. EIF2AK3 encoded PERK is an ER-stress regulator activated when cells encounter metabolic stress. Thus, EBNA3C mediated autophagy activation provides an alternative energy source through degradation of K63-linked polyubiquitinated protein aggregates for aberrant cell proliferation and subsequent B-cell lymphoma development.